## REMARKS

Reconsideration of the subject application in view of the present amendment is respectfully requested.

By the present amendment, the specification has been amended to define the weight unit of the weight of the claimed hyperpolymeric hemoglobin and to define its size in comparison with quarteznary hemoglobin. Claim 6 has been amended to more precisely define the present invention.

Based on the forgoing amendments and the following remarks, the application is deemed to be in conditions for allowance and action to that end is respectfully requested.

### I. Rejection of Claims.

Ia. In Rejection of Claims Under 35 U.S.C. § 112, First Paragraph.

The Examiner rejected claims 6-10 under 35 U.S.C. § 112, First paragraph, for not being supported by an enabling disclosure. Specifically, the Examiner pointed out that the specification has not provided any written guidance as to the definition of the weight and size of the hyperpolymeric

hemoglobin claimed. With regard to weight, the attention of the Examiner is respectfully directed to examples 1-4 in which the weight of the hyperpolymeric hemoglobin is listed. Further, as noted above, the specification has been amended to indicate the weight unit of the weight of the hyperpolymeric gemoglobin given in the section "Summary of the Invention."

As to the size of the hyperpolymeric gemoglobin, it is within the knowledge of one or ordinary skill in the art. The size of the hyperpolymeric hemoglobin can be defined by using the volume excluding chromatography, as discussed in the Pötzschke et al. article cited by the Examiner. By this method the hyperpolymeric molecules are separated according to their molecular hydrodynamic volume, and this volume is in a specific relationship to the molecular weight of the molecules. As can be seen from present examples 1 to 3 or 4, the hydrodynamic volume is about ten times to about (5-10) x 100 times of that of the hemoglobin. This results from the values as indicated in the present examples, which show as an upper limit of 500,000 up to 15 x $10^5$ g per mol. Since hemoglobin (not crosslinked) has the value of 65,000 g per mol, the relation as indicated in present claim 6 is up to (5-10) x100 times the size of hemoglobin, because of the size

exclusion of the chromatographic material in relation to the molecular weight as indicated.

This is the result obtained by the presently used analytical volume excluding chromatography as mentioned above and explained in Pötzschke. According to that method, molecules, as already explained, as separated by the volume they can have when passing the analytical chromatography column.

It is respectfully submitted that the amendment to the specification indicating the size of the crosslinked hyperpolymeric hemoglobin does not constitute new maller as it is within knowledge of ordinary skill in the art, and is cased on the original disclosure (examples).

Ib. Rejection under 35 U.S.C. § 112, Second Paragraph

The Examiner rejected claims 6-10 under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. Specifically, the Examiner pointed out that the size of the hyperpolymeric hemoglobin molecule in comparison with quaternary hemoglobin cannot be defined. It is respectfully

submitted that the amendment of claim 6 overcomes the Examiner rejection under 35 U.S.C. § 112, second paragraph.

Ic. Rejection of Claims Over the Prior Art

The Examiner rejected claims 6-8 under 35 U.S.C. § 102(b) as being anticipated by Pötzschke et al. 1992 Article (Pötzschke) and rejected claims 6-10 under 35 U.S.C. § 103(a) as being unpatentable over Pötzschke in view of Bonhard et al. U.S. Patent No. 4,136,093 (Bonhard).

It is respectfully submitted that claims 6-10 are patentable over the cited prior art.

The Examiner is correct that Pötzschke describes the cross-linking of hemoglobin with glutaraldehyde to obtain hyperpolymeric hemoglobin. However, Pötzschke does not describe a purification of the product obtained. Under "Materials and Methods", page 289, it is said that an analytical sample has been taken, and it has been disclosed that a stabilized hemoglobin, as prepared according to that reference, does not show changes in molecular weight distribution, see page 290 under "Results." Thereby , only small amounts of the hemoglobin solution are analyzed by that

method, see for example page 289, last line, from which the inner diameter of the column, as used, can be taken which is 1 cm. According to the claimed method the column, as used, has a diameter of 2.6 cm and a flow of 27 ml/h, whereas the flow of the analytical method according to Pötzschke is only 5.5 ml/h (see page 290, first line). That means that in the present case, a preparative method is used whereas according to the Pötzschke, only an analytical method is used. Since hyperpolymeric hemoglobins have not been investigated with regard to their size and molecular weight distribution up to the present invention (_____ see the references as cited in the present description), it could not have been foreseen that a method directed to an analytical scale can easily be transformed to a preparative scale the more so as it has been known at the time of filing the present invention that the hyperpolymeric hemoglobins are frequently not separated as a whole since they are large chain molecules in contrast to the usually available proteins, see page 7, last paragraph up to page 8, 2nd paragraph. Thus, Pötzschke does not reveal the chromatographic fractionation, but only analytical investigations. Moreover, Pötzschke only cites fractionation with regard to ultrafiltration (see page 290, under "Results") which however, is completely different from chromatography and not subject matter of the present claims. Thus, from the

state of the art it could not have been expected and therefore has surprisingly been found out that by chromatographying a cross-linked hyperpolymeric hemoglobin solution of a large scale, molecularly uniform hyperpolymeric hemoglobins can be obtained.

In view of the above, it is respectfully submitted that claim 6 is not anticipated by PÖtzschke and is patentable thereover.

The Examiner has also rejected the present invention in view of Bonhard which would teach a method of cross-linking hemoglobin and then diminishing the amount of uncross-linked hemoglobin using ammonium sulfat. Therefore, the Examiner concludes that it would have been obvious to one of ordinary skilled in the art to use ammonium sulfat solution as presently done, applicants respectfully disagree.

The Examiner correctly notes that Bonhard discloses the cross-linking of hemoglobin. Bonhard also discloses the use of ammonium sulfat. However, Bonhard's intention is to separate known cross-linked hemoglobin from uncross-linked hemoglobin. Therefore, Bonhard uses a diluted mixture of cross-linked and uncross-linked hemoglobin solution to which

ammonium sulfate is added, and which then is stirred for only some minutes (see example 5 of the Bonhard reference from which these parameters can be taken). In the present case, however, on one hand, a more concentrated solution of the hyperpolymeric hemoglobins is taken (3.5%, see present example 2) and, on the other hand, the reaction with ammonium sulfate is carried out for a much more longer time (e.g., 4, 5 hours, see present example 2). Thus, the conditions as presently used are completely different from those as applied by Bonhard.

Moreover, the aim according to the present invention was to provide a method to separate molecularly uniform hemoglobin hyperpolymers from a known hemoglobin hyperpolymer solution having a broad distribution of different molecular weights whereby at the same time a pharmaceutically acceptable low viscosity as required for clinical use is obtained, see page 6, last paragraph up to page 7, 2nd paragraph. The object of Bonhard, however, is to provide for a hemoglobin preparation with increased oxygen release. Such an object is achieved by a condensation product of hemoglobin and pyridoxal phosphate. Thus, not only the conditions with regard to the treatment with ammonium sulfate are different, but also the objects of Bonhard and the presently claimed method are

different. Consequently, the one skilled in the art when reading the Bonhard reference could not find there any suggestiion of how to prepare a cross-linked hemoglobin solution having a uniform molecular size and weight distribution and moreover having such a low viscosity that it can still be used for clinical applications. Thus, it would not be obvious to one of ordinary skill in the art to use ammonium sulfat solution because this solution would precipitate any uncross-linked remaining hemoglobin in the obtained cross-linked product, since the object of the present invention was not to obtain a purer cross-linked product but a solution being composed and useful, as mentioned above.

In view of the above, it is respectfully submitted that neither Pötzschke not Bonhard render the claimed subject matter obvious since, on one hand, they have different objects and, on the other hand, different reaction conditions exist. Such conditions are not disclosed in the art and could not have been developed by routine experimentation since there is only little knowledge as regards size and weight distribution of cross-linked hemoglobins.

In view of the above, it is respectfully submitted that claim 6 patentably defines over the prior art and is, therefore, allowable.

Claims 7-10 depend on claim 6 and are allowable for the same reason claim 6 is allowable and further because of specific features recited therein which, when taken above and/or in combination with features recited in claim 6, are not disclosed or suggested in the prior art.

**CONCLUSION**

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance, and allowance of the application is respectfully requested.

Should the Examiner require or consider it advisable that the specification, claims and/or drawings be further amended or corrected in formal respects, in order to place the case in condition for final allowance, then it is respectfully requested that such amendment or correction be carried out by Examiner's Amendment and the case passed to issue. Alternatively, should the Examiner feel that a personal discussion might be helpful in advancing this case to

allowance, the Examiner is invited to telephone the undersigned.

Respectfully submitted
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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on March 17, 1998.

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